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(57) Abstract

There are provided DNA (or RNA) polynucleotide sequences encoding naturally occurring splice variants of human growth hormone, hGHV-2 (88) and hGHV-3 (53) as well as analogs and derivatives thereof, which both lack nucleotide sequences normally present in the gene which codes for wild-type human growth hormone. The growth hormone variants of the present invention are of human origin and are useful for diagnostic, preventative and therapeutic purposes with respect to certain human diseases. The present invention is also related to a method for producing the human growth hormone variants by recombinant DNA techniques. A method of generating an antibody directed against and therefore inhibiting the activity of wild-type growth hormone is also provided.

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HUMAN GROWTH HORMONE

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are naturally occurring splice variants of human growth hormone, hGHV-2(88) and hGHV-3(53). The invention also relates to inhibiting the action of such polypeptides.

Human growth hormone is produced in and released from the pituitary gland. hGHwt is a peptide hormone having several useful functions. One characteristic activity of human growth hormone is that it directs linear bone growth. Human growth hormone also has lactogenic activity and has been shown to be involved in metabolic processes such as lipid, nitrogen and carbohydrate metabolism. Recent studies on human growth hormone have begun to suggest that different regions of the molecule might be involved in controlling the above stated activities. Other indications of human growth hormone include its use in the treatment of hip fractures in the elderly, chronic renal insufficiency, Turner's syndrome, cancer and HIV infection and its subsequent effects.

Considerable effort has been expended in studying the human growth hormone molecule and its interaction with other cells and organs of the human body in an attempt to regulate human growth hormone's effect on these particular targets in the human body. For example, wild-type human growth hormone (hGHwt) is presently used to treat hypopituitism where not enough human growth hormone occurs naturally. Accordingly, a means by which to stimulate the natural production of human growth hormone in hypopituitism or to compete with the receptor sites of human growth hormone when a patient is subjected to hyperpituitism would be of great value, particularly in light of the fact that certain growth abnormalities are capable of being diagnosed prenatally.

Means and methods for production of human growth hormone variant proteins and to the determination of their DNA sequences and amino acid sequence of 191 amino acids by recombinant DNA technology were disclosed in U.S. Patent No. 4,670,393 issued to Seeburg on June 2, 1987, however, hitherto the present invention, there has not been disclosed two naturally occurring spliced variants of the human growth hormone polypeptide.

The polypeptides of the present invention has been putatively identified as human growth hormone splice variants. This identification has been made as a result of amino acid sequence homology with wild type human growth hormone (hHGwt).

In accordance with one aspect of the present invention, there are provided novel mature polypeptides which are naturally occurring splice variants of the human growth hormone, hGHV-2(88) and hGHV-3(53), as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules

encoding hGHV-2(88) and hGHV-3(53), including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments thereof.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing hGHV-2(88) or hGHV-3(53) nucleic acid sequences, under conditions promoting expression of said proteins and subsequent recovery of said protein.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotide encoding such polypeptides for therapeutic purposes, for example, stimulating the action of wild-type hGH and for inhibiting the action of wild-type hGH.

In accordance with yet a further aspect of the present invention, there is also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to human hGHV-2(88) and hGHV-3(53) sequences.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with yet another aspect of the present invention, there are provided antagonists to such polypeptides, which may be used to inhibit the action of such polypeptides, for example, in the treatment of hyperpituitism.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1 is a schematic view of the polypeptides of wild-type human growth hormone, variant hGHV-2(88) and variant hGHV-3(53).

FIG. 2(a) shows the cDNA and corresponding deduced amino acid sequence of the wild-type hGH and Figure 2(b) shows the cDNA and corresponding deduced amino acid sequence corresponding to hGHV-2(88) with Figure 2(c) showing the cDNA and corresponding deduced amino acid sequence of hGHV-3(53). The standard one-letter abbreviations for amino acids are used. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.). Sequencing accuracy is predicted to be greater than 97% accurate.

FIG. 3 illustrates a gel after bacterial expression and purification of clones containing the coding sequences for wild-type hGH, hGHV-2(88) and hGHV-3(53).

FIG. 4 illustrates the ability of the purified proteins shown in FIG. 3 to bind to hGH receptors normally present on IM-9 cells.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptides having the deduced amino acid sequences of Figure 2 (SEQ ID No. 5 and 6) or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75600 and 75601 for on November 3, 1993 for hGHV-2(88) and hGHV-3(53), respectively.

Polynucleotides encoding polypeptides of the present invention may be obtained from pituitary tissue. They are structurally related to hGHwt and contain open reading frames encoding mature polypeptides of 172 and 151 amino acids. They are completely homologous to hGHwt except for the amino acids deleted as a result of alternative mRNA splicing. Variant hGHV-2(88) and variant hGHV-3(53) are generated by

alternative splicing of the pre-mRNA where the splice donor site of exon-2 is fused to two alternate sites within exon-3. This results in the removal of 57 and 120 nucleotides, respectively.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequences which encode the mature polypeptides may be identical to the coding sequences shown in Figure 2 (SEQ ID No. 5 and 6) or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides as the DNAs of Figure 2 (SEQ ID No. 2 and 3) or the deposited cDNAs.

The polynucleotides which encode for the mature polypeptides of Figure 2 (SEQ ID No. 5 and 6) or for the mature polypeptides encoded by the deposited cDNAs may include, but is not limited to: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequences of Figure 2 (SEQ ID No. 5

and 6) or the polypeptides encoded by the cDNAs of the deposited clones. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figure 2 (SEQ ID No. 5 and 6) or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figure 2 (SEQ ID No. 5 and 6) or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figure 2 (SEQ ID No. 2 and 3) or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence

is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 2 (SEQ ID No. 2 and 3) or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of

Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to polypeptides which have the deduced amino acid sequence of Figure 2 (SEQ ID No. 5 and 6) or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figure 2 (SEQ ID No. 5 and 6) or that encoded by the deposited cDNAs, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptides of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptides of Figure 2 (SEQ ID No. 5 and 6) or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature

polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating

promoters, selecting transformants or amplifying the hGHV-2(88) and hGHV-3(53) genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic

trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spondoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-DEXTRAN mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable

prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise

an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides of this invention can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

hGHwt directs linear bone growth, has lactogenic activity and has also been shown to be involved in metabolic processes, such as lipid, nitrogen and carbohydrate metabolism, and has also been used to treat hip fractures in the elderly, chronic renal insufficiency, Turner's syndrome, cancer and AIDS.

Recent studies on wild-type human growth hormone have suggested that different regions of the molecule might be involved in directing these different activities.

hGHV-2(88) binds to growth hormone receptors and produces a downstream response similar to that of hGHwt. In this way hGHV-2(88) is an agonist to hGHwt. Conversely, hGHV-3(53) also binds to the receptors however, no such downstream response is produced. In this way, hGHV-3(53) acts to occupy receptor sites preventing activity normally associated with hGHwt. Therefore, hGHV-3(53) is an antagonist to hGHwt.

Accordingly, hGHV-2(88) could be used to treat hypopituitism by specifically stimulating production of hGHwt which then directs linear bone growth, since hypopituitism is characterized by abnormally slow growth and results in dwarfism. In the same manner, hGHV-2(88) could be used to increase milk production in cattle by stimulating lactogenic activity. hGHV-2(88) may also be used to treat obesity due to its possible lipolytic activity, to break down lipid in fat cells. In the same manner, hGHV-2(88) may be used to achieve the other functions of hGHwt, namely, carbohydrate metabolism to result in muscle production, treatment of hip fractures in the elderly, treatment of chronic renal insufficiency, Turner's syndrome, cancer and AIDS.

The human growth hormones splice variants of the present invention have fewer amino acids than the hGHwt. Accordingly, the human growth hormone variants may retain certain activities which are characteristic of hGHwt while not retaining others. Therefore the human growth hormone variants can be used to increase one function of hGHwt without increasing another. Accordingly, it is within the scope of this invention to identify and produce alternatively spliced human growth hormone variants which have specific functions of hGHwt while not having others.

hGHV-3(53) may be employed to treat hyperpituitism since hGHV-3(53) competes with hGHwt for the normal hGHwt receptor sites. By this action the normal functions of hGHwt are slowed or prevented altogether. hGHV-3(53) therefore is useful for treating gigantism and acromegaly which result from a hyperactive pituitary gland.

Fragments of the full length hGHV-2(88) and hGHV-3(53) genes may be used as a hybridization probe for a cDNA library to isolate the full length hGHV-2(88) and hGHV-3(53) genes and to isolate other genes which have a high sequence similarity to the hGHV-2(88) and hGHV-3(53) genes or similar biological activity. Probes of this type generally have at least 20 bases. Preferably, however, the probes have at least 30 bases and generally do not exceed 50 bases, although they may have a greater number of bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete hGHV-2(88) and hGHV-3(53) genes including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the hGHV-2(88) and hGHV-3(53) genes by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to human disease.

This invention provides a method for identification of the receptors for hGHV-2(88) and hGHV-3(53). The gene encoding the receptors can be identified by by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). Preferably,

expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the hGHV-2(88) and hGHV-3(53) ligands, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to them. Transfected cells which are grown on glass slides are exposed to labeled hGHV-2(88) and hGHV-3(53). hGHV-2(88) and hGHV-3(53) can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and rescreening process, eventually yielding a single clone that encodes the putative receptor(s).

As an alternative approach for receptor identification, labeled hGHV-2(88) and hGHV-3(53) can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the ligand-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

The polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 10 μg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The hGHV-2(88) and hGHV-3(53) polypeptides which are polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art and are apparent from the teachings herein. For example, cells may be engineered by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. For example, a packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells nmay be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention.

This invention is also related to the use of the hGHV-2(88) and hGHV-3(53) genes as a diagnostic. Detection of a mutated form of these genes will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of hGHV-2(88) and hGHV-3(53).

Individuals carrying mutations in the human hGHV-2(88) and hGHV-3(53) genes may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding hGHV-2(88) and hGHV-3(53) can be used to identify and analyze mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA or alternatively, radiolabeled antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between the reference gene and genes having mutations may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamidine gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The present invention also relates to a diagnostic assay for detecting altered levels of hGHV-2(88) and hGHV-3(53) protein in various tissues since an over-expression of the proteins compared to normal control tissue samples can detect the presence of these proteins. Assays used to detect levels of hGHV-2(88) and hGHV-3(53) protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the hGHV-2(88) and hGHV-3(53) antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, flourescence or in this example a horseradish peroxidase enzyme. A sample is now removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like BSA. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any hGHV-2(88) and hGHV-3(53) proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to hGHV-2(88) and hGHV-3(53). Unattached reporter antibody is then washed out. Peroxidase substrates are tehn added to the dish and the amount of color developed in a given time preiod is a measurement of the amount of hGHV-2(88) and hGHV-3(53) protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to hGHV-2(88) and hGHV-3(53) is attached to a solid

support and labeled hGHV-2(88) and hGHV-3(53) and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of hGHV-2(88) and hGHV-3(53) in the sample.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the express sequence tag (EST) was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and purification of the hGHV-2(88) and hGHV-3(53)

The DNA sequences encoding for HGHV-2(88) and hGHV-3(53) (ATCC No.s 75600 and 75601, respectively) are initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer has the sequence 5' AGAGGATCCGCCATGGCTACAGGCTCCCGG 3', (SEQ ID No. 7) contains a BamHI restriction enzyme site followed by 21 nucleotides of hGHV-2(88) and hGHV-3(53) coding sequence starting from the initiation codon; the 3' sequence contains complementary sequences to T7 promoter/sequence in the CDVA cloning vector, a translation stop codon and the hGHV-2(88) and hGHV-3(53) coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen Inc., Chatsworth, CA). The plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 vector was digested with BamHI and SalI and the insertion fragments were then ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. Figure 5 shows a schematic representation of this arrangement. The ligation mixture was then used to transform the *E. coli* strain available from Qiagen under the trademark m15/rep4. M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates containing both Amp and Kan. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in either LB media supplemented with both Amp (100 μg/ml) and Kan (25 μg/ml).

The O/N culture is used to inoculate a large culture at a dilution of 1:100 to 1:250. The cells were grown to an optical density of 600 ($O.D._{600}$) between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3-4 hours. Cells were then harvested by centrifugation (20' at 6000Xg). The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCL. After clarification, solubilized hGH variants were purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag. (Hochuli, E. et al., Genetic Engineering, Principle & Methods, 12:87-98 (1990). hGHV-2(88) and hGHV-3(53) (95% pure) were eluted from the column in 6 molar guanidine HCL pH 5.0. Protein renaturation out of Guanidine HCl can be accomplished by several protocols. (Jaenicke, R. and Rudolph, R. Protein Structure - A Practical Approach IRL Press, New York (1990)). Initially, step dialysis is utilized to remove the GnHCl. Alternatively, the purified protein isolate from the Ni-chelate column can be bound to a second column over which a decreasing linear GnHCl gradient is run. The protein is allowed to renature while bound to the column and is subsequently eluted with GnHCl, pH 5.0. Finally, soluble protein is dialyzed against a storage buffer containing 140mM NaCl, 20mM $NaPO_4$ and 10% w/v Glyconol.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ROSEN, ET AL.

(ii) TITLE OF INVENTION: Human Growth Hormone

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/187,756
(B) FILING DATE: 27 JAN 1994

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-55

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 654 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGGCTGCAG GCTCCCGGAC GTCCCTGCTC CTGGCTTTTG GCCTGCTCTG CCTGTCCTGG   60
CTTCAAGAGG GCAGTGCCTT CCCAACCATT CCCTTATCCA GGCTTTTTGA CAACGCTATG  120
CTCCGCGCCC GTCGCCTGTA CCAGCTGGCA TATGACACCT ATCAGGAGTT TGAAGAAGCC  180
TATATCCTGA AGGAGCAGAA GTATTCATTC CTGCAGAACC CCCAGACCTC CTCTGCTTC   240
TCAGAGTCTA TTCCAACACC TTCCAACAGG GTGAAAACGC AGCAGAAATC TAACCTAGAG  300
CTGCTCCGCA TCTCCCTGCT GCTCACTCAG TCATGGCTGG AGCCCGTGCA GCTCCTCAGG  360
AGCGTCTTCG CCAACAGCCT GGTGTATGGC GCCTCGGAGA GCAACGTCTA TCGCCACCTG  420
AAGGACCTAG ACGAAGGCAT CCAAACGCTG ATGTGGAGGC TGGAAGATGG CAGCCCCCGG  480
ACTGGGCAGA TCTTCAATCA GTCCTACAGC AAGTTTGACA CAAAATCGCA CAACGATGAC  540
GCACTGCTCA AGAACTACGG GCTGCTCTAC TGCTTCAGGA AGGACATGGA CAAGGTCGAG  600
ACATTCCTGC GCATCGTGCA GTGCCGCTCT GTGGAGGGCA GCTGTGGCTT CTAG         654
```

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 597 BASE PAIRS
(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATGGCTGCAG GCTCCCGGAC GTCCCTGTCT CTGGCTTTTG GCTCGCTCTG CCTGTCCTGG   60
CTTCAAGAGG GCAGTGCCTT CCCAACCATT CCCTTATCCA GGCTTTTTGA CAACGCTATG  120
CTCCGCGCCC GTCGCCTGTA CCAGCTGGCA TATGACACCT ATCAGGAGTT TTCCCTCTGC  180
TTCTCAGAGT CTATTCCAAC ACCTTCCAAC AGGGTGAAAA CGCAGCAGAA ATCTAACCTA  240
GAGCTGCTCC GCATCTCCCT GCTGCTCACT CAGTCATGGC TGGAGCCCGT GCAGCTCCTC  300
AGGAGCGTCT TCGCCAACAG CCTGGTGTAT GGCGCCTCGG AGAGCAACGT CTATCGCCAC  360
CTGAAGGACC TAGAGGAAGG CATCCAAAGC CTGATGTGGA GGCTGGAAGA TGGCAGCCCC  420
CGGACTGGGC AGATCTTCAA TCAGTCCTAC AGCAAGTTTG ACACAAAATC GCACAACGAT  480
GACGCACTGC TCAAGAACTA CGGGCTGCTC TACTGCTTCA GGAAGGACAT GGACAAGGTC  540
GAGACATTCC TGCGCATCGT GCAGTGCCGC TCTGTCGAGG GCAGCTGTGG CTTCTAG     597
```

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 534 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGGCTGCAG GCTCCCGGAC GTCCCTGCTC CTGGCTTTTG GCCTGCTCTG CCTGTCCTGG   60
CTTCAAGAGG GCAGTGCCTT CCCAACCATT CCCTTATCCA GGCTTTTTGA CAACGCTATG  120
CTCCGCGCCC GTCGCCTGTA CCAGCTGGCA TATGACACCT ATCAGGAGTT TAACCTAGAG  180
CTGCTCCGCA TCTCCCTGCT GCTCACTCAG TCATGGCTGG AGCCCGTGCA GCTCCTCAGG  240
AGCGTCTTCG CCAACAGCCT GGTGTATGGC GCCTCGGACA GCAACGTCTA TCGCCACCTG  300
AAGGACCTAG AGGAAGGCAT CCAAACGCTG ATGTGGAGGC TGGAAGATGG CAGCCCCCGG  360
ACTGGGCAGA TCTTCAATCA GTCCTACAGC AAGTTTGACA CAAAATCGCA CAACGATGAC  420
GCACTGCTCA AGAACTACGG GCTGCTCTAC TGCTTCAGGA AGGACATGGA CAAGGTCGAG  480
ACATTCCTGC GCATCGTGCA GTGCCGCTCT GTGGAGGGCA GCTGTGGCTT CTAG        534
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 217 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Ala Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu
   -25                 -20                 -15
Leu Cys Leu Ser Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile
  -10                  -5                   1
Pro Leu Ser Arg Leu Phe Asp Asn Ala Ser Leu Arg Ala His Arg
5                     10                  15
Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala
20                    25                  30
Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro Gln
35                    40                  45
Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50                    55                  60
Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser
65                    70                  75
Leu Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg
80                    85                  90
Ser Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn
95                   100                 105
Val Tyr Asp Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu
110                  115                 120
Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe
125                  130                 135
Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp
140                  145                 150
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Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp
155                 160                 165
Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser
170                 175                 180
Val Glu Gly Ser Cys Gly Phe
185                 190
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 198 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Ala Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu
   -25                 -20                 -15
Leu Cys Leu Ser Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile
   -10                 -5                   1
Pro Leu Ser Arg Leu Phe Asp Asn Ala Ser Leu Arg Ala His Arg
 5                   10                  15
Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Ser Leu Cys
20                  25                  30
Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln
35                  40                  45
Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Leu Ile
50                  55                  60
Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala
65                  70                  75
Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu
80                  85                  90
Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
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95                  100                 105
Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr
110                 115                 120
Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys
125                 130                 135
Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val
140                 145                 150
Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser
155                 160                 165
Cys Gly Phe
170
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 177 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Ala Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu
   -25                 -20                 -15
Leu Cys Leu Ser Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile
  -10                  -5                   1
Pro Leu Ser Arg Leu Phe Asp Asn Ala Ser Leu Arg Ala His Arg
5                   10                  15
Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Asn Leu Glu
20                  25                  30
Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser Trp Leu Glu Pro
35                  40                  45
Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val Tyr Gly
```

```
50                  55                  60
Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Glu
65                  70                  75
Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg
80                  85                  90
Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn
95                  100                 105
Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr
110                 115                 120
Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile
125                 130                 135
Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
140                 145                 151
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGAGGATCCG CCATGGCTAC AGGCTCCCGG 30

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide having the deduced amino acid sequence of SEQ ID No. 5 or a fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding a polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75600 or a fragment, analog or derivative of said polypeptide.

(a) a polynucleotide encoding a polypeptide having the deduced amino acid sequence of SEQ ID No. 6 or a fragment, analog or derivative of said polypeptide; and

(b) a polynucleotide encoding a polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75601 or a fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 5.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide having the deduced amino acid sequence of SEQ ID No. 6.

7. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide encoded by the cDNA of ATCC Deposit No. 75600.

8. The polynucleotide of Claim 2 wherein said polynucleotide encodes a polypeptide encoded by the cDNA of ATCC Deposit No. 75601.

9. The polynucleotide of Claim 1 having the coding sequence as shown in SEQ ID No. 2.

10. The polynucleotide of Claim 1 having the coding sequence as shown in SEQ ID No. 3.

11. The polynucleotide of Claim 2 having the coding sequence deposited as ATCC Deposit No. 75600.

12. The polynucleotide of Claim 2 having the coding sequence deposited as ATCC Deposit No. 75601.

13. A vector containing the DNA of Claim 2.

14. A host cell genetically engineered with the vector of Claim 13.

15. A process for producing a polypeptide comprising: expressing from the host cell of Claim 14 the polypeptide encoded by said DNA.

16. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 13.

17. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having hGHV-2(88) activity.

18. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having hGHV-3(53) activity.

19. A polypeptide selected from the group consisting of: (i) a polypeptide having the deduced amino acid sequence of SEQ ID No. 5 and fragments, analogs and derivatives thereof; (ii) a polypeptide encoded by the cDNA of ATCC Deposit No. 75600 and fragments, analogs and derivatives of said polypeptide; (iii) a polypeptide having the deduced amino acid sequence of SEQ ID No. 6 and fragments, analogs and derivatives thereof; and (iv) a polypeptide encoded by the cDNA of ATCC Deposit No. 75601 and fragments, analogs and derivatives of said polypeptide.

20. The polypeptide of Claim 19 wherein the polypeptide has the deduced amino acid sequence of SEQ ID No. 5.

21. The polypeptide of Claim 19 wherein the polypeptide has the deduced amino acid sequence of SEQ ID No. 6.

22. An antibody against the polypeptide of claim 19.

23. A method for the treatment of a patient having need of human growth hormone activity comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 20.

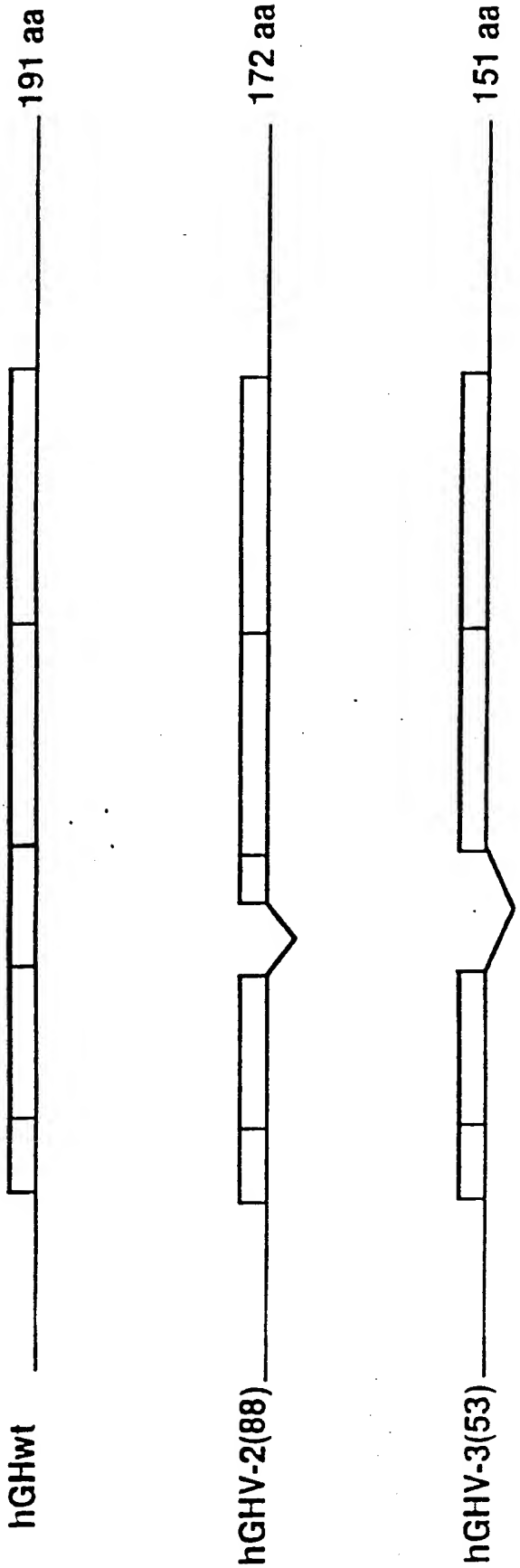
24. A method for the treatment of a patient having need to inhibit human growth hormone activity comprising: administering to the patient a therapeutically effective amount of the antagonist of Claim 21.

25. The method of Claim 23 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

26. The method of Claim 24 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

FIG. 1

Schematic of hGH Splice Variants



Human Growth Hormone Splice Variants

FIG. 2Aa

MATCH WITH FIG. 2Ab

hGHwt

```
-26
 M   A   A   G   S   R   T   S   L   L   L   L   A   F
ATG GCT GCA GGC TCC CGG ACG TCC CTG CTC CTG CTG GCT TTT—→
  1
 F   P   T   I   P   L   S   R   L   F   D   N   A
TTC CCA ACC ATT CCC TTA TCC AGG CTT TTT GAC AAC GCT—→

                32
 T   Y   Q   E   F   E   E   A   Y   I   P   K   E   —→
ACC TAT CAG GAG TTT GAA GAA GCC TAT ATC CTG AAG GAG
 C   F   S   E   S   I   P   T   P   S   N   R   E
TGC TTC TCA GAG TCT ATT CCA ACA CCT TCC AAC AGG GTG—→
 S   L   L   L   I   Q   S   W   L   E   P   V   Q
TCC CTG CTG CTC ACT CAG TCA TGG CTG GAG CCC GTG CAG—→
 A   S   D   S   N   V   Y   D   L   L   K   D   L
GCC TCG GAC AGC AAC GTC TAT CGC CAC CTG AAG GAC CTA—→
 G   S   P   R   T   G   Q   I   F   K   Q   T   Y
GGC AGC CCC CGG ACT GGG CAG ATC TTC AAT CAG TCC TAC—→
 L   K   N   Y   G   L   L   Y   C   F   R   K   D
CTC AAG AAC TAC GGG CTG CTC TAC TGC TTC AGG AAG GAC—→

                191
 R   S   V   E   G   S   C   G   F
CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG
```

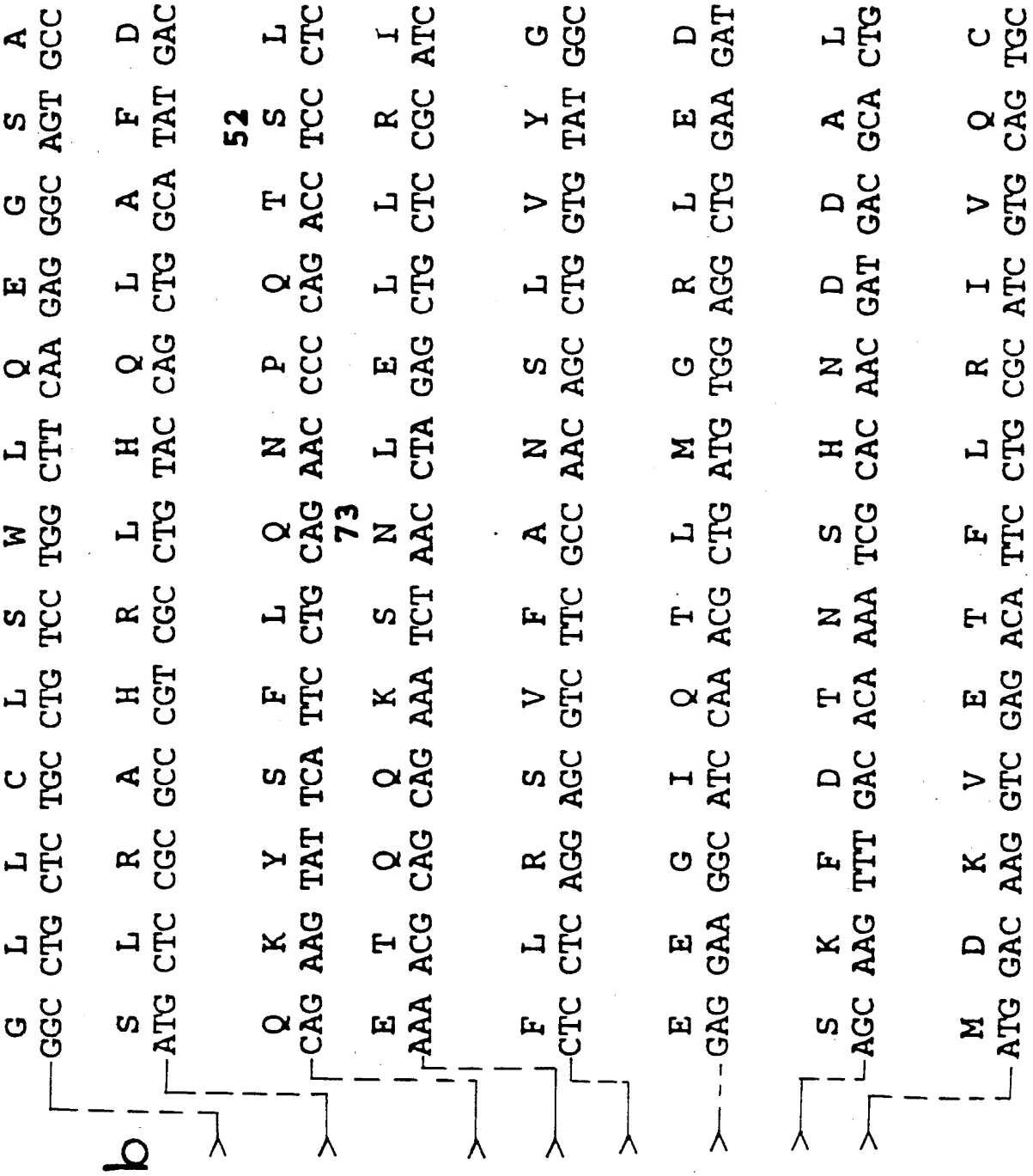


FIG. 2Ab

MATCH WITH FIG. 2Aa

FIG. 2Ba

hGHV-2(88)

```
-26
 M  A  A  G  S  R  T  S  L  L  L  A  F
ATG GCT GCA GGC TCC CGG ACG TCC CTG CTC CTG GCT TTT→

1
 F  P  T  I  P  L  S  R  L  F  D  N  A
TTC CCA ACC ATT CCC TTA TCC AGG CTT TTT GAC AAC GCT→

                32
 T  Y  Q  E  F
ACC TAT CAG GAG TTT ... ... ... ... ... ... ... ...→

 C  F  S  E  S  I  P  T  P  S  N  R  E
TGC TTC TCA GAG TCT ATT CCA ACA CCT TCC AAC AGG GTG→

 S  L  L  L  I  Q  S  W  L  E  P  V  Q
TCC CTG CTG CTC ACT CAG TCA TGG CTG GAG CCC GTG CAG→

 A  S  D  S  N  V  Y  D  L  L  K  D  L
GCC TCG GAC AGC AAC GTC TAT CGC CAC CTG AAG GAC CTA→

 G  S  P  R  T  G  Q  I  F  K  Q  T  Y
GGC AGC CCC CGG ACT GGG CAG ATC TTC AAT CAG TCC TAC→

 L  K  N  Y  G  L  L  Y  C  F  R  K  D
CTC AAG AAC TAC GGG CTG CTC TAC TGC TTC AGG AAG GAC→
                                172

 R  S  V  E  G  S  C  G  F
CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG
```

MATCH WITH FIG. 2Bb

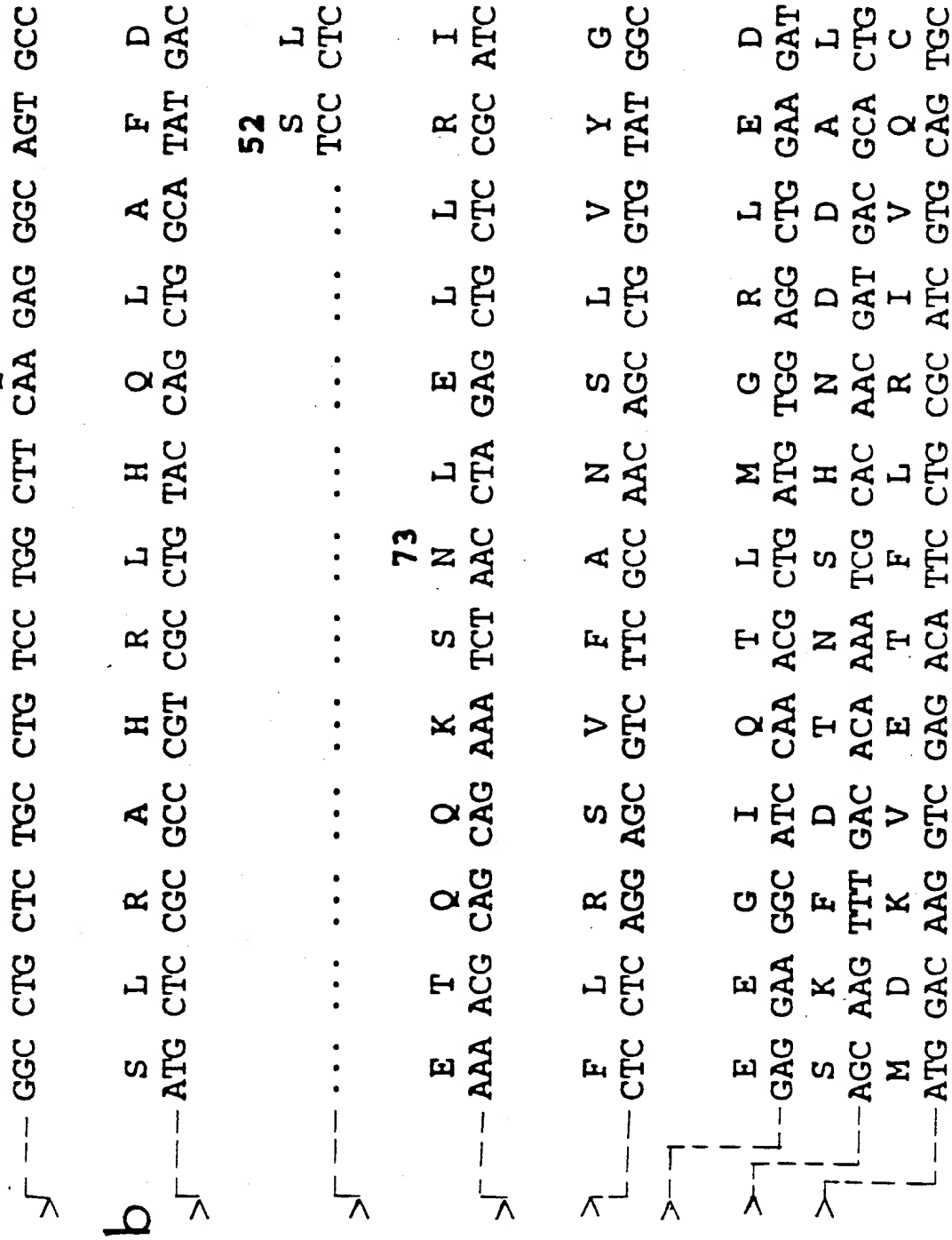


FIG. 2Bb

MATCH WITH FIG. 2Ba

hGHV-3(53)

```
-26
 M   A   A   G   S   R   T   S   L   L   L   A   F
ATG GCT GCA GGC TCC CGG ACG TCC CTG CTC CTG GCT TTT→

  1
 F   P   T   I   P   L   S   R   L   F   D   N   A
TTC CCA ACC ATT CCC TTA TCC AGG CTT TTT GAC AAC GCT→

                32
 T   Y   Q   E   F
ACC TAT CAG GAG TTT ... ... ... ... ... ... ... ...→

... ... ... ... ... ... ... ... ... ... ... ... ...→
 S   L   L   L   I   Q   S   W   L   E   P   V   Q
TCC CTG CTG CTC ACT CAG TCA TGG CTG GAG CCC GTG CAG→
 A   S   D   S   N   V   Y   D   L   L   K   D   L
GCC TCG GAC AGC AAC GTC TAT CGC CAC CTG AAG GAC CTA→
 G   S   P   R   T   G   Q   I   F   K   Q   T   Y
GGC AGC CCC CGG ACT GGG CAG ATC TTC AAT CAG TCC TAC→
 L   K   N   Y   G   L   L   Y   C   F   R   K   D
CTC AAG AAC TAC GGG CTG CTC TAC TGC TTC AGG AAG GAC→

                                151
 R   S   V   E   G   S   C   G   F
CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG
```

FIG. 2Ca

MATCH WITH FIG. 2Cb

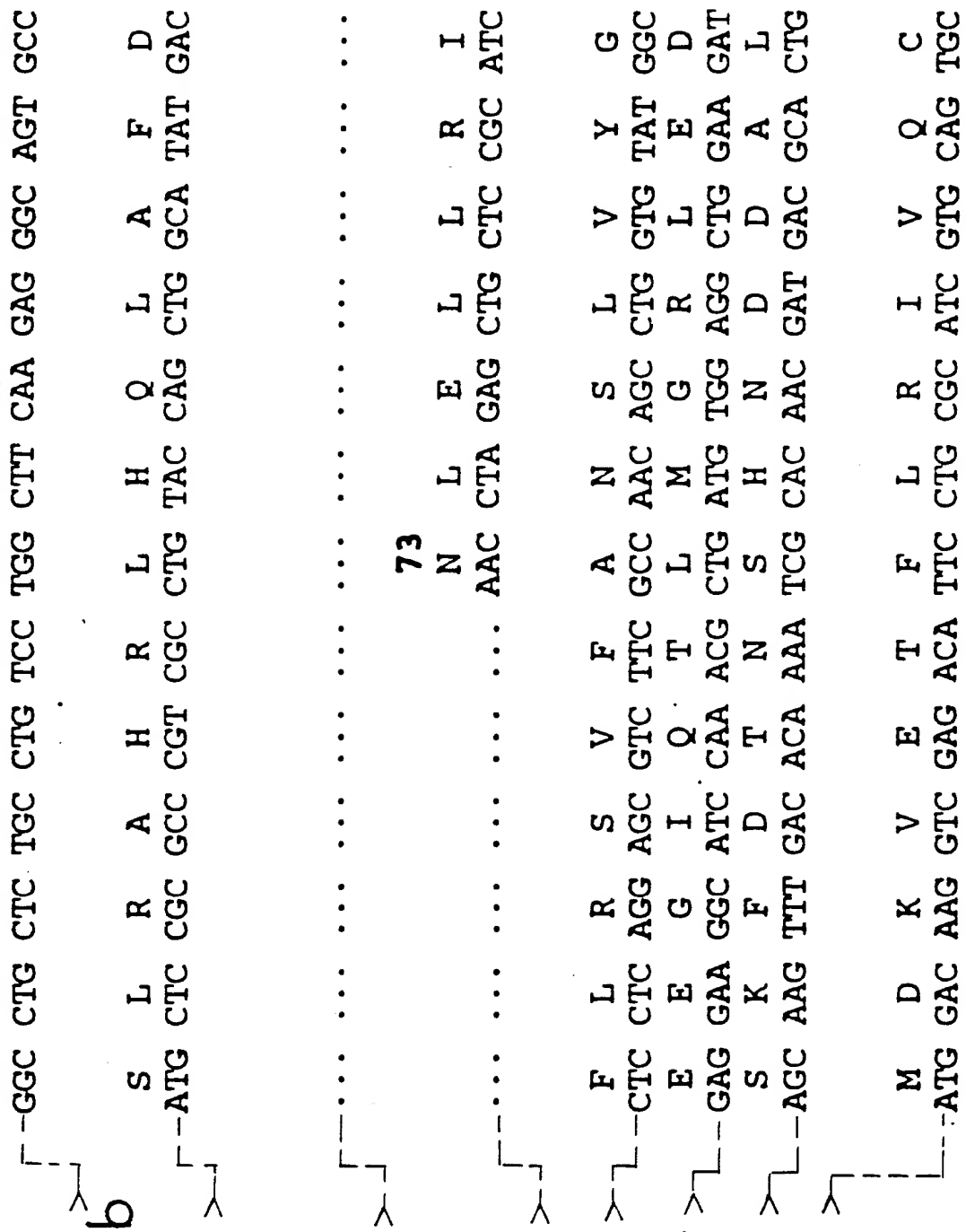


FIG. 2Cb

MATCH WITH FIG. 2Ca

FIG. 3

Bacterial Expression and Purification of hGH Variants

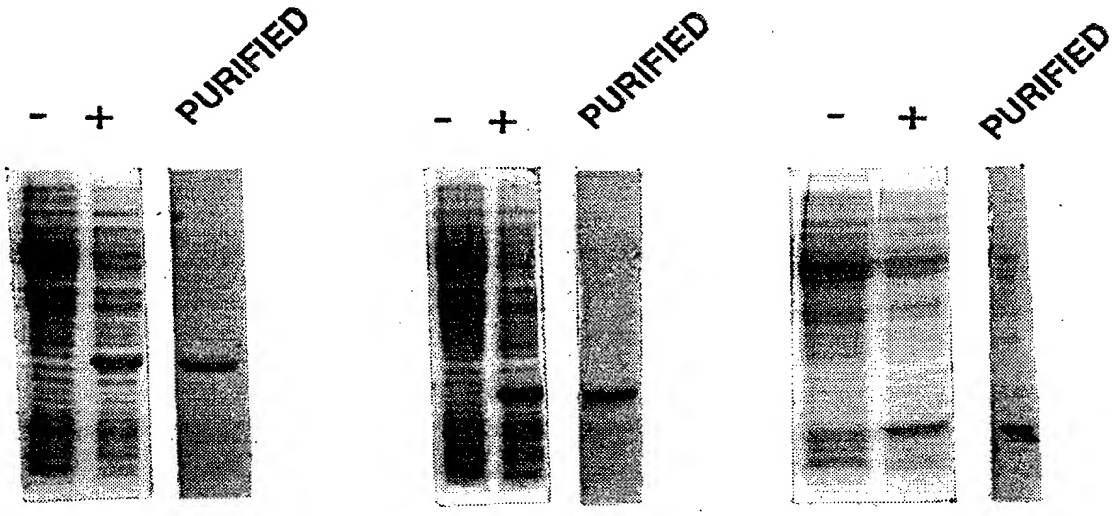
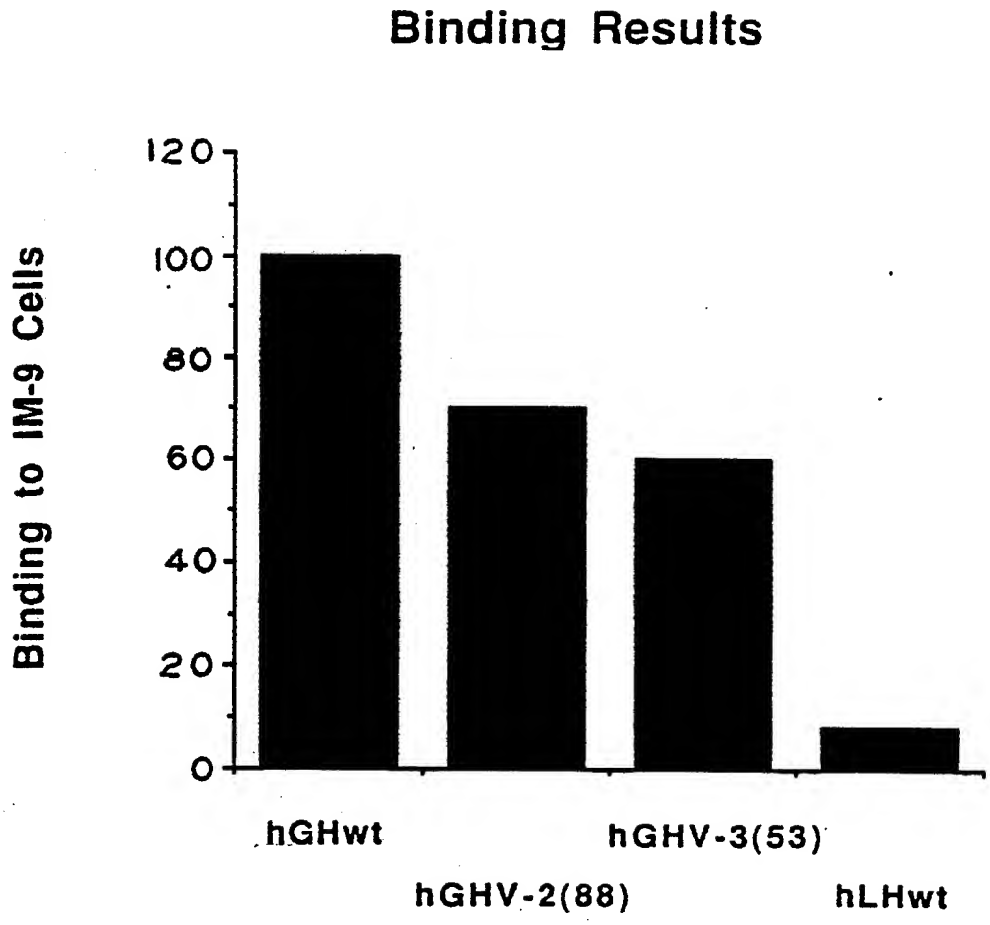


FIG. 4


Binding Results
Binding to IM-9 Cells
120
100
80
60
40
20
0
hGHwt
hGHV-3(53)
hGHV-2(88)
hLHwt

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01130

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/22, 38/27; C12Q 1/00; C07H 21/02, 21/04; C07K 14/61; C12N 1/00, 5/10, 15/18
US CL :435/69.4, 240.1, 252.3, 320.1; 530/397, 399; 536/23.1, 23.51; 514/2, 8, 12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.4, 240.1, 252.3, 320.1; 530/397, 399; 536/23.1, 23.51; 514/2, 8, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG - Biotech Files, GenEMBL sequence databases

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Biological Chemistry, Volume 265, Number 32, issued 15 November 1990, P. A. Estes et al, "A difference in the splicing patterns of the closely related normal and variant human growth hormone gene transcripts is determined by a minimal sequence divergence between two potential splice-acceptor sites", pages 19863-19870, see entire document.	1-4, 13-16, 19
X	Journal of Biological Chemistry, Volume 267, Number 21, issued 25 July 1992, P. A. Estes et al, "A native RNA secondary structure controls alternative splice-site selection and generates two human growth hormone isoforms", pages 14902-14908, see entire document.	1-4, 13-16, 19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 MAY 1995	Date of mailing of the international search report 02 JUN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELIZABETH C. KEMMERER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01130

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Clinical Investigation, Volume 82, issued July 1988, N. E. Cooke et al, "Human growth hormone gene and the highly homologous growth hormone variant gene display different splicing patterns", pages 270-275, see entire document.	1-4, 13-16, 19
X	US, A, 4,446,235 (SEEBURG ET AL) 01 May 1984, columns 1-14, see entire document.	1-4, 13-16, 19
X	US, A, 4,670,393 (SEEBURG ET AL) 02 June 1987, columns 1-14, see entire document.	1-4, 13-16, 19
X	Science, Volume 205, issued 1979, J. A. Martial et al, "Human growth hormone: complementary DNA cloning and expression in bacteria", pages 602-606, see entire document.	1-4, 13-16, 19
X	Philosophical Transactions of the Royal Society of London Biology, Volume 307, issued 1984, R. L. Brinster et al, "Transgenic mice containing growth hormone fusion genes", pages 309-312, see entire document.	1-4, 13-16, 19
X	Journal of Biological Chemistry, Volume 265, Number 14, issued 15 May 1990, J. Ray et al, "Human growth hormone-variant demonstrates a receptor binding profile distinct from that of normal pituitary growth hormone", pages 7939-7944, see entire document.	1-4, 13-16, 19
X	Endocrinology, Volume 126, Number 2, issued 1990, B. E. Nickel et al, "The human placental growth hormone variant is mitogenic for rat lymphoma Nb2 cells" pages 971-976, see entire document.	1-4, 13-16, 19
X	Molecular and Cellular Endocrinology, Volume 91, Numbers 1/2, issued 1993, B. E. Nickel et al, "Differential expression of human placental growth hormone variant and chorionic somatomammotropin genes in choriocarcinoma cells treated with methotrexate", pages 159-166, see entire document.	1-4, 13-16, 19
X	Nucleic Acids Research, Volume 7, Number 2, issued 1979, W. G. Roskam et al, "Molecular cloning and nucleotide sequence of the human growth hormone structural gene", pages 305-320, see entire document.	1-4, 13-16, 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01130

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proceedings of the National Academy of Sciences USA, Volume 76, issued 1979, J. C. Fiddes et al, "Structure of genes for human growth hormone and chorionic somatomammotropin", pages 4294-4298, see entire document.	1-4, 13-16, 19
X	Journal of Biological Chemistry, Volume 263, Number 18, issued 25 June 1988, N. E. Cooke et al, "Two distinct species of human growth hormone-variant mRNA in the human placenta predict the expression of novel growth hormone proteins", pages 9001-9006, see entire document.	1-4, 13-16, 19

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-21, 23, and 24, drawn to DNA, proteins, methods of preparing proteins, and methods of administering proteins.

II. Claim 22, drawn to an antibody.

III. Claims 25 and 26, drawn to gene therapy.

and it considers that the International Application does not comply with the requirements of unity of invention (PCT Rules 13.1, 13.2, and 13.3) for the reasons indicated below:

The antibody of Group II does not share a special technical feature with the DNA and protein of Group I, and can also be used in materially different methods. For example, the DNA and protein can be used therapeutically whereas the antibody can be used in a diagnostic kit. The antibody of Group II is not required for the gene therapy methods of Group III. Finally, the DNA and proteins of Group I do not share a special technical feature with the gene therapy methods of Group III because the DNA can be used to make the protein which can be used in traditional therapy rather than in the gene therapy methods. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.